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The Hypothalamic-Pituitary-Adrenocortical System

Clinical Evaluation by Pharmacologic Techniques

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■ *The hypothalamic-pituitary-adrenocortical system is a complex negative feed-back control mechanism. Under non-stress conditions it functions to maintain a circadian rhythm of adrenal steroid production which is secondary to a circadian fluctuation in the sensitivity to suppression of* ACTH *by hydrocortisone. Any stressful stimulus causes an augmentation of steroid production which is due in part to a decrease in sensitivity of* ACTH *to suppression. Aldosterone production is not primarily controlled by* ACTH.

The increased understanding of this system and of its pathologic alterations has led to the development of a number of pharmacologic techniques which have proved most valuable in evaluating its integrity. Hypothalamic-pituitary function can be assessed by the response to the administration of dexamethasone, methopyrapone, bacterial pyrogen and vasopressin. Adrenal cortical function can be assessed by the response to ACTH, *dexamethasone and spironolactone.*

FOR MANY YEARS, it has been known that there is a fundamental "push-pull" relationship between the pituitary gland and the adrenal gland which serves to control the output of corticosteroids from the adrenal. Only recently, however, has the complexity of this relationship come to be appreciated. New information has given new insights and has made possible additional diagnostic techniques which have proved to be most helpful in diagnosing abnormalities of this system. Many of the newer diagnostic tests measure the response to the administration of a pharmacologic agent. The purpose of this review is to consider the controlling mechanisms and to discuss pharmacologic techniques used to determine their integrity.

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Controlling Mechanisms

The essence of the pituitary-adrenal relationship is a negative feedback system. If the concentration of circulating adrenal steroids in the plasma tends to fall, ACTH is released by the pituitary gland in increased quantities. ACTH stimulates the adrenal

gland to elaborate a greater quantity of steroids so that equilibrium is reestablished. However, as the production of adrenal steroids continues, further release of ACTH by the pituitary is inhibited and this serves to avoid overcompensation by an excessive production of steroids.

It is appreciated that such a simple feedback control system would function to keep the concentration of adrenal corticosteroids relatively constant in the blood at all times. However, if one measures the concentration of steroids in the plasma, one finds that the level does not remain constant, but fluctuates through a cycle of 24 hours' duration. The greatest adrenal activity occurs in the very early hours of the morning and it diminishes slowly and progressively until there is only minimal activity in the late hours of the evening, resulting in what has come to be called the circadian rhythm of steroid secretion. (*Circadian* means "about the day" and appears to be a desirable replacement for the term *diurnal,* which has approximately the same meaning but which unfortunately sounds more like a urologic term.) Recent studies indicate that the circadian steroid secretion pattern is secondary to a circadian secretion pattern of ACTH, or more properly to a circadian fluctuation in the sensitivity to suppression of ACTH by hydrocortisone.[15]

The nature of the stimuli which initiate this basic rhythm are not completely understood. That the circadian rhythm is not simply secondary to the ocular stimulus of daylight is indicated by the fact that blind persons evidence the same rhythmic output of corticosteroids as do sighted individuals. Since persons who work night shifts appear to have a pattern of steroid secretion similar to those who work day shifts, it would seem that physical activity is not the initiating stimulus. However, if a person moves from one part of the world to another, his rhythmic pattern of steroid secretion (after a short period of adjustment) assumes the cycle of others living at the same longitude.

It should be mentioned that of the various adrenal secretory products, only hydrocortisone, the important glucocorticoid secreted by the human adrenal cortex, is responsible for suppressing ACTH secretion. It is normally produced in quantities of approximately 20 mg a day. All other naturally-occurring steroids are relatively ineffective in producing such suppression. All of the potent synthetic steroids do effectively suppress ACTH. Aldosterone, the important mineralocorticoid of the human adrenal cortex, is as potent, milligram for milligram, as hydrocortisone in suppressing ACTH; but since it is only produced in quantities of approximately 100 mcg per day, it is biologically unimportant in this regard.

To this point we have considered only the production of adrenal steroids under normal nonstress conditions. It is well known, however, that under circumstances of maximum stress the production of hydrocortisone by the adrenal gland can reach levels 10 times the normal baseline output. This response to stress would seem to be caused, in part at least, by a stress-induced change in the sensitivity of ACTH suppressibility. It has been demonstrated, for instance, that if a person undergoing a relatively minor surgical procedure such as a hemorrhoidectomy (it should be noted that this is not often considered a minor procedure by the patient) is given an amount of hydrocortisone slightly in excess of that which he would normally produce in such circumstances, there is no increase in ACTH production and consequently no increase in endogenous hydrocortisone production. This indicates that during stress of this order, ACTH can still be suppressed; however, the circulating levels of hydrocortisone necessary to effect suppression in the stressed state are much higher than those normally circulating in the unstressed state. If, however, a person undergoes an operation such as a gastrectomy (considered by all to be a major surgical procedure), then it has been shown that the administration of hydrocortisone in quantities greatly in excess of those which would normally be produced under such circumstances fail completely to suppress ACTH elaboration.

Formerly it was thought that suppression of ACTH by hydrocortisone as well as its increased production secondary to stress was mediated by the pituitary gland. There is now a great deal of evidence from a number of sources to indicate that both of these effects on the pituitary take place through the hypothalamus, which acts as an essential intermediate in a sort of double-play combination consisting of adrenal-to-hypothalamus-to-pituitary.

It will be recalled that the blood supply to the anterior pituitary gland is somewhat different from the blood supply to most of the tissues of the body. The anterior pituitary receives the predominate portion of its blood from a large venous channel which courses down the pituitary stalk. This is a true portal system, in that this blood comes di-

rectly from a capillary network originating in the area of the hypothalamus. There is convincing evidence that specific peptides called "corticotropin-releasing factors," produced by cells in the hypothalamus, are carried by the "portal system" to the pituitary gland and play a major role in controlling the release of ACTH. One of these peptides has been particularly well characterized and has been found to be very similar in its molecular composition to antidiuretic hormone or vasopressin.[6]

It should be mentioned in passing, that the production of aldosterone is influenced, but not primarily controlled through ACTH action; rather it is controlled through an increased generation of angiotensin acting on the adrenal cortex.[2]

With this framework of the structure of the system controlling adrenal cortical function in mind, it is possible to understand some of the pharmacologic procedures used by endocrinologists in the clinical evaluation of the integrity of the system.

Assessing Hypothalamic-Pituitary Function

The detection of abnormalities in the ability of the pituitary gland to properly produce ACTH has been compromised, to say the least, by the lack of a simple assay procedure with sufficient sensitivity to demonstrate subnormal levels of ACTH. Without such an assay it has been necessary to rely heavily on pharmacologic methods of eliciting an ACTH response and to measure an increase in adrenal secretory products in the patient's blood or urine as a reflection of that response.

In 1959, Liddle[10] and his associates demonstrated that pituitary function could be tested by the administration of methopyrapone or SU-4885. The latter term, which was the manufacturer's code designation for the compound, unfortunately has persisted despite the fact that it sounds very much like a telephone number and is approximately as difficult to remember. Methopyrapone blocks adrenal synthesis of hydrocortisone, but does not block the production of other steroids. In a normal subject, if hydrocortisone cannot be produced, ACTH secretion is not inhibited through the negative feedback mechanism. Under the influence of increased ACTH secretion there is an increased production of adrenal steroids other than hydrocortisone, and these are measured by the commonly used urinary steroid methods. In normal persons therefore, the administration of methopyrapone produces an increase in urinary steroid secretion, but in persons with disruption of the hypothalamic-pituitary relay system such an increase does not occur. The methopyrapone test is a means for determining the integrity of hypothalamic-pituitary unit in regard to its function in the negative feedback control system. The normal response to the oral administration of methopyrapone, 500 mg every six hours for two or three days, is a two-fold to three-fold increase in urinary steroids. The failure for such a response to occur in a patient who has intact adrenals as demonstrated by a suitable response to ACTH administration, is presumptive evidence of hypothalamic-pituitary dysfunction.[10]

Another method for assessing the ability of the hypothalamic-pituitary unit to produce an increase in ACTH is to measure the urinary steroid response to the stressful stimulus provided by an injection of bacterial pyrogen. Unfortunately, certain untoward symptoms must be provoked in order to achieve a definite adrenal response, and for this reason this test does not have wide clinical acceptance.

Our studies of the adrenal response to the injection of a pharmacologic dose of synthetic vasopressin suggested that such a response might be due to the similarity between vasopressin and of corticotropin-releasing factors,[8] and that vasopressin might serve as a suitable stand-in for corticotropin-releasing factors (until these factors are available for clinical use) in assessing the ability of the pituitary to release ACTH. We found that in normal subjects, the intramuscular injection of 10 units of synthetic vasopressin consistently produced a two-fold increase in the concentration of plasma hydrocortisone one hour following the injection while patients with pituitary tumors had a diminished response or none at all.[9] In a limited number of patients we found a similar response when commercially available vasopressin was used instead of synthetic vasopressin.

Detecting Adrenal Insufficiency

Since ACTH became commercially available, testing the integrity of the adrenal cortex has been a relatively easy task. The standard procedure consists of the intravenous administration of ACTH.[4] The increased production of hydrocortisone is usually assessed by measuring either urinary 17-hydroxy steroids or urinary ketogenic steroids, and the increased production of adrenal androgen by measuring urinary 17-ketosteroids. The adrenal production of androgens as well as hydrocortisone is controlled to a large extent by ACTH. In males, of course, under normal circumstances, approxi-

mately one-third of the urinary 17-ketosteroids are derived from androgen produced in the testicle, which is not under the control of ACTH. With the standard infusion test, ACTH is administered intravenously over either six or eight hours daily for four or five days. Although a definite response can generally be appreciated by the first or second day of stimulation, it is sometimes advisable to continue for a full five days in order to rule out the presence of "relative" adrenal insufficiency. In such a case, urinary steroids may increase for the first day or two, but subsequently, as the reserve capacity of the adrenal is exceeded, their secretion may diminish again in the latter days of the test.[17] A normal adrenal gland should continue to respond to the challenge and to increase progressively its output of steroids each day for five days.

It is not generally appreciated that the number of units of ACTH used in the infusion is greatly in excess of the maximal effective dose. More than 2 units over an eight-hour period gives no increased adrenal response, and is added only to serve as a most generous safety factor or out of deference to convention. The time over which the infusion is given is important, however. Each additional hour of ACTH administration produces a greater response.

Since a quantitative response is not generally necessary in establishing a clinical diagnosis of adrenal insufficiency, the procedure for stimulating the adrenal has been modified in order to avoid the necessity of intravenous infusions. Under such circumstances, a slowly absorbed preparation of ACTH, such as corticotropin-zinc, is injected intramuscularly every 12 hours for four or five days and 24-hour urinary specimens are assayed for adrenal steroids. When ACTH is given intramuscularly, it is generally necessary to use larger quantities of ACTH (such as 20 units every 12 hours) since it is not as active when it is administered by this route.

In 1965 we suggested a simplified screening test for adrenal insufficiency[12] which obviated the necessity of intravenous infusion and urine collections. In this test, 25 units of aqueous ACTH is administered intramuscularly and, one hour after injection, the plasma-hydrocortisone level is assayed fluorometrically and compared with the plasma-hydrocortisone level immediately preceding the injection. Normally, the post-injection concentration is more than double the pre-injection concentration. This has proved in our experience to be a reliable screening procedure with the obvious advantage of the very short duration of the test and elimination of the need for collecting an accurate 24-hour urine specimen, which is always difficult.

If, however, there is any doubt about the interpretation of the results of either test in which ACTH is administered by the intramuscular route, it is advisable to repeat the procedure, using daily intravenous infusions of ACTH.

Any patient in whom a diagnosis of adrenal insufficiency is under serious consideration should be given a small daily dose of a potent synthetic glucocorticoid such as dexamethasone, 1.0 mg on the day before and the same amount on each day of ACTH administration. This does not influence in any way the results of the test and appears to offer protection against the untoward serious reactions to ACTH which may occur in patients with adrenal insufficiency.

Demonstrating Adrenal Hyperplasia and Tumors

The excessive production of adrenal steroids may result from either of two conditions: An adrenal tumor (benign or malignant), or bilateral adrenal hyperplasia.

An adrenal tumor producing excessive quantities of hydrocortisone and/or androgens is by definition autonomous and not under the control of the hypothalamic-pituitary system. In other words, the tumor does not stop producing steroids when ACTH levels fall, and, in point of fact, if the tumor makes hydrocortisone excessively (some tumors secrete mainly androgens) then ACTH is continuously suppressed. Such adrenal tumors generally produce high 24-hour urinary steroid levels which are not lowered by the administration of large dosages of dexamethasone (2 mg every six hours) which assures complete suppression of ACTH.[11] Therefore, in any case in which the administration of dexamethasone in such dosage for several days fails to bring about a decided reduction in urinary steroids, the presence of an adrenal tumor is suspected.

Other adrenal tumors produce aldosterone as their only important secretory product. These tumors cause high blood pressure and although they have been previously regarded as an unusual cause of hypertension, Conn[3] recently produced evidence indicating that such tumors are responsible for a significant proportion of cases of so-called "essential" hypertension. Conn showed that such patients may have perfectly normal serum electrolytes and

can therefore not be distinguished from other persons with hypertension by simple electrolyte determination. The detection of patients with this curable form of hypertension has now become a difficult and demanding problem.

The diagnosis of an aldosterone-producing tumor in a hypertensive patient with normal electrolytes can be made by demonstrating depressed serum renin activity following the application of appropriate physiologic stimuli. Such procedures, however, can only be accomplished in a few research laboratories.[3] We have recently shown that patients with aldosterone-producing tumors can be more easily detected by a simple pharmacologic test.[7] We have found that, in such patients, normalization of blood pressure is predictable after several weeks of treatment with spironolactone, 50 mg four times daily. The striking response in blood pressure to spironolactone contrasts decidedly with the minimal effect on blood pressure of hydrochlorthiazide, 50 mg twice daily, administered during a subsequent four-week control period. Patients with hypertension not caused by an adrenal tumor characteristically show only a moderate response to either spironolactone or hydrochlorothiazide, and the greater effect is usually produced by the thiazide.

Bilateral adrenal hyperplasia (Cushing's syndrome) is the result of an excessive or inappropriate secretion of ACTH.[5,18] Occasionally it may result from the production of an ACTH-like substance by a malignant neoplasm of diverse origin.[1,13] Such cases will not be considered in this review. Nelson[14] and his associates have shown that in early or mild cases of Cushing's syndrome there is only a loss of the increased sensitivity of hypothalamic-pituitary suppressibility to hydrocortisone which normally occurs in the latter part of each day so that the circadian rhythm of steroid secretion is lost. The concentration of serum hydrocortisone in such cases may not be outside of normal limits in the early hours, but the expected fall later in the day does not occur.[2] In more advanced cases, the resistance of the hypothalamus and pituitary to suppression is even further increased and the concentration of serum hydrocortisone is consistently above normal levels. The "thermostat" is set too high but it is still operating, and although the resistance of ACTH to suppression is increased, suppression can still be achieved with a higher than normal concentration of hydrocortisone. This is similar to the way in which the heat can be turned off in an overheated room by burning a candle under the thermostat. Liddle[11] and coworkers defined this relationship and devised a clinical application for candle-burning which has proved to be a most valuable pharmacologic test for distinguishing patients with Cushing's syndrome from normal or obese subjects (obese subjects characteristically have higher urinary steroids and they are continually being considered as possible cases of a "glandular disorder") and from patients with hydrocortisone producing adrenal tumors.

Liddle showed that the administration of dexamethasone, 0.5 mg every six hours for several days, consistently caused a pronounced decrease in urinary steroid secretion in normal persons but did not produce a significant reduction in the urinary steroids of patients with Cushing's syndrome. The subsequent increase of the dosage of dexamethasone to 2.0 mg every six hours, however, did cause suppression of steroid secretion in patients with adrenal hyperplasia, but not in patients with adrenal tumors.

More recently, Pavatos, Smilo and Forsham[16] suggested a similar but abbreviated test designed simply to separate patients with adrenal hyperplasia or tumors from normal and obese subjects. They showed that the administration of a single dose of dexamethasone, 1.0 mg orally at 11:00 p.m., will cause a predictable depression of the concentration of plasma 17-hydroxycorticosteroids the following morning in normal and obese subjects. Patients with adrenal hyperplasia or tumor are resistant to suppression when this procedure is used.

In the future our ability to evaluate the integrity of the hypothalamic-pituitary-adrenocortical system will be greatly improved by the development of assays for ACTH which are more sensitive and, it is to be hoped, more simple. Similar assays for corticotropin-releasing factors, and angiotensin would be most welcome additions to our diagnostic box of tools. The availability of synthetic corticotropin-releasing factors would lead to the development of other pharmacologic techniques which might revolutionize our ability to evaluate ACTH production. In the meantime, the correlation of the results of the various available pharmacologic techniques may help us to better localize the functional lesion in individual patients and lead to an increased understanding of the function of the hypothalamic-pituitary-adrenocortical system in man.

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